PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/155

A1

(11) International Publication Number: **WO 97/45108**

(43) International Publication Date: 4 December 1997 (04.12.97)

(21) International Application Number: PCT/US97/08280

(22) International Filing Date: 16 May 1997 (16.05.97)

(30) Priority Data:
08/655,631 30 May 1996 (30.05.96) US

(71) Applicant: CHILDREN'S HOSPITAL MEDICAL CENTER [US/US]; 3333 Burnet Avenue, Cincinnati, OH 45229 (US).

(72) Inventors: SOUTHAN, Garry, J.; 406 Navaho Drive, Fredrick, MD 21701 (US). SALZMAN, Andrew, L.; 8280 Kugler Mill Road, Cincinnati, OH 45243 (US). SZABO, Csaba; 3654 Stonebridge Drive #E, Cincinnati, OH 45233 (US).

(74) Agents: JOSEPHIC, David, J. et al.; Wood, Herron & Evans, L.L.P., 2700 Carew Tower, Cincinnati, OH 45202 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GUANIDINO DERIVATIVES AS INHIBITORS OF THE CYTOTOXIC EFFECT OF PEROXYNITRITE

(57) Abstract

This invention is directed to a pharmacologically acceptable composition for inhibiting the cytotoxic effect of peroxynitrite in a mammal, which includes a guanidino derivative and a pharmaceutically acceptable carrier. The invention also concerns a method of inhibiting the cytotoxic effect of peroxynitrite, and treating various conditions where there is an advantage in inhibiting the cytotoxic effect of peroxynitrite. The method includes the step of administering to a mammal a guanidino derivative in pure form or in a pharmaceutically acceptable carrier.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

GUANIDINO DERIVATIVES AS INHIBITORS OF THE CYTOTOXIC EFFECT OF PEROXYNITRITE

Background of the Invention

The present invention relates to the use of guanidino derivatives as inhibitors of the cytotoxic effect of peroxynitrite.

Peroxynitrite is a reactive oxidant produced from nitric oxide (NO) and superoxide, which reacts with proteins, lipids and DNA under conditions of inflammation and shock. Immunohistochemical and biochemical evidence demonstrate production of peroxynitrite in endotoxic and hemorrhagic shock, chronic bowel inflammation, and in various forms of ischemia-reperfusion injury. The reactivity and decomposition of peroxynitrite is determined by the chemical environment, and the ratio of superoxide vs. NO. Peroxynitrite can initiate toxic oxidative reactions *in vitro* and *in vivo*. Initiation of lipid peroxidation, direct

inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na^+/K^+ ATP-ase activity, inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of peroxynitrite. In addition, peroxynitrite is a potent trigger of DNA strand breakage, with subsequent activation of the nuclear enzyme poly-ADP ribosyl synthetase (PARS), with eventual severe energy depletion of the cells.

There is now good experimental evidence that inhibition of the formation of peroxynitrite and/or scavenging peroxynitrite is beneficial in a number of pathophysiological conditions, including circulatory shock and ischemia-reperfusion injury. Theoretically, peroxynitrite-mediated cytotoxicity can be prevented in a number of ways. First, inhibition of nitric oxide biosynthesis would reduce the formation of nitric oxide, and, consequently, of peroxynitrite. Second, scavenging superoxide or enhancing its decomposition (with agents such as superoxide dismutase) also would reduce the production of peroxynitrite. A third approach would be the use of agents which would directly inhibit the oxidative reactions and cytotoxic effect triggered by peroxynitrite. While various inhibitors of the first two approaches have been patented and proposed for therapeutic use, the available tools to directly inhibit peroxynitrite-induced oxidative processes is limited.

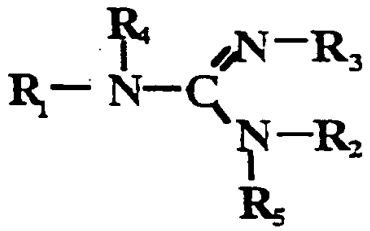
Therefore, it would be extremely beneficial to have a composition and/or method for directly inhibiting peroxynitrite's oxidative reactions and cytotoxic effect.

Summary of the Invention

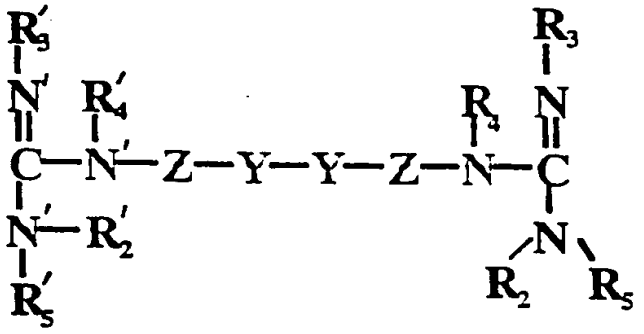
This invention is directed to a pharmacologically acceptable composition for inhibiting the cytotoxic effect of peroxynitrite in a mammal. The composition includes a guanidino derivative and a pharmaceutically acceptable carrier, with the guanidino derivative present in the composition in an effective amount to inhibit the cytotoxic effect of peroxynitrite in the mammal.

The invention also is directed to a method of inhibiting the cytotoxic effect of peroxynitrite in a mammal, which includes the step of administering to the mammal a guanidino derivative in a pure form or in a pharmaceutically acceptable carrier.

The guanidino derivative of the composition and method is defined by a formula selected from the group consisting of:



and



or a salt thereof, wherein:

R_2 and R'_2 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, aminoalkyl, hydroxy, alkoxy, thioalkylene, thioesteralkylene, phenyl or phenylalkylene, or a substituted derivative thereof;

R_3 and R'_3 are independently H, lower alkyl, alkylene, alkenylene, amino, hydroxy, thioalkylene, or a substituted derivative thereof;

R_1, R_5, R'_5, R_4 and R'_4 are independently H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene or amino, or a substituted derivative thereof;

Alternatively, R_1 is R_6-Y-Z- where R_6 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene, acyl, $-SO_3^-$, or $-PO_3^-$, or a substituted derivative thereof, and Z and Y are as defined below;

Z and Z′ are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

Y and Y′ are independently S or Se;

When R_2 or R'_2 is alkylene, alkenylene, thioalkylene, amino, hydroxy or a substituted derivative thereof, said R_2 or R'_2 may be joined to any of:

(i) R_3 or R'_3, if R_3 or R'_3 is alkylene, alkenylene or thioalkylene;

(ii) R_4 or R'_4, if R_4 or R'_4 is alkylene or alkenylene; or

(iii) R_5 or R'_5, if R_5 or R'_5 is alkylene or alkenylene;

to form 5-, 6-, or 7-membered heterocycle;

When R_2, R_3, R'_2 or R'_3 is alkylene or alkenylene, said R_2, R_3, R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6-membered heterocyclic ring, with the proviso that said heterocyclic ring optionally is substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

When R_1 and R_4 are alkylene or alkenylene, said R_1 and R_4 optionally may be joined together to form a 5-, 6-, or 7-membered heterocycle;

When R_1 is R_6-Y-Z-, and R_6 is alkylene or alkenylene, R_6 optionally may be joined to any of:

(i) R_2, when R_2 is alkylene, alkenylene or thioalkylene;

(ii) Z; or

(iii) R_4, when R_4 is alkylene or alkenylene;

to form a 5-, 6- or 7-membered heterocyclic ring; and

a pharmaceutically acceptable carrier, said compound present in said composition in an effective amount to inhibit the cytotoxic effect of peroxynitrite in said mammal.

As used herein, the term "salt" refers to any addition salt derived from any pharmaceutically acceptable organic or inorganic acid. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene p sulfonic, tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulphonic acids. Additionally, as used herein, any alkyl or alkylene may be straight chain, branched or cyclic, and "halo" includes bromine, chlorine, fluorine and iodine.

In the descriptions mentioned above, a substituted derivative of R_2, R_3, R'_2 and R'_3 refers to a derivative that may be substituted with one or more alkoxy, halo, hydroxy and amino groups. A substituted derivative of R_1, R_5, R'_5, R_4 and R'_4 refers to a derivative that may be substituted with one or more alkyl, alkoxy, halo, hydroxy, amino, amino alkyl (secondary or tertiary), thio and nitro groups.

If the R_2, R'_2, R_3 or R'_3 substituent is thioalkylene, the thioalkylene preferably has a formula [-$(CH_2)_n$-SH] where n is independently 1 to 4. If the R_2, or R'_2 substituent is thioesteralkylene, the thioesteralkylene preferably has the formula

$[-(CH_2)_n-S-R_7]$ where R_7 is independently a lower alkyl and n is independently 1 to 4.

The Z and Z' substituents of the guanidino derivative are independently alkylene, alkenylene, cycloalkyene or cycloalkenlyene, or a substituted derivative thereof. When such a substituted derivative is employed, the substituent may include one or more of lower alkyl, alkoxy, halo, amino, nitro or carboxyl.

Brief Description of the Drawings

Fig. 1 is a graph of the inhibitory effect of mercaptoethylguanidine (MEG), S-methyl-mercaptoethylguanidine (SMEG), guanidinoethyldisulfide (GED) and aminoguanidine (AG) on the oxidation of dihydrorhodamine 123 (DHR) by peroxynitrite;

Fig. 2A is a graph of the protective effect of aminoguanidine (AG) on mitochondrial respiration in J774 macrophages exposed to peroxynitrite;

Fig. 2B is a graph of the protective effect of mercaptoethylguanidine (MEG), on mitochondrial respiration in J774 macrophages exposed to peroxynitrite;

Fig. 2C is a graph of the protective effect of guanidinoethyldisulfide (GED) on mitochondrial respiration in J774 macrophages exposed to peroxynitrite; and

Fig. 3 is a graph showing the protective effect of aminoguanidine (AG), mercaptoethylguanidine (MEG) or

guanidinoethyldisulfide (GED) against the DNA single strand breakage induced by peroxynitrite.

Fig. 4 is a graph showing the protective effect of mercaptoethylguanidine (MEG) on the peroxynitrite-induced suppression of vascular contractility.

Detailed Description of the Invention

This invention is directed to a pharmacologically acceptable composition for inhibiting the cytotoxic effect of peroxynitrite in a mammal. The composition includes a guanidino derivative and a pharmaceutically acceptable carrier, with the guanidino derivative present in the composition in an effective amount to inhibit the cytotoxic effect of peroxynitrite in the mammal. The invention also is directed to a method of inhibiting the cytotoxic effect of peroxynitrite in a mammal, which includes the step of administering to the mammal a guanidino derivative in pure form or in a pharmaceutically acceptable carrier.

Suitable guanidino derivatives for use in the composition or method are made according to the methods of synthesis taught in the following articles which are incorporated herein in their entirety by reference: (1) Joseph X. Khym et al., "Ion Exchange Studies of Transguanylation Reactions. I. Rearrangement of S,2-Aminoethylisothiourea to 2-Mercaptoethylguanidine and 2-Aminothiazoline", *Journal of the American Chemical Society*,

Vol. 79, pp. 5663-5666, November 5, 1957; (2) David G. Doherty, et al., "Synthesis of Aminoalkylisothiuronium Salts and their Conversion to Mercaptoalkylguanidines and Thiazolines", *Journal of the American Chemical Society*, Vol. 79, pp. 5667-5671, November 5, 1957; (3) Joseph X. Khym, et al., "Ion Exchange Studies of Transguanylation Reactions. II. Rearrangement of 3-Aminopropylisothiourea and N-Substituted Aminoethyl- and Aminopropylisothioureas to Mercaptoalkylguanidines and 2-Aminothiazolines or Penthiazolines", *Journal of the American Chemical Society*, Vol. 80, pp. 3342-3349, July 5, 1958; (4) David G. Doherty et al. "Synthesis of D- and L-2-Aminobutylisothiourea Dihydrobromide Isomers and their Conversion to Guanidothiols, Disulfides, and Thiazolines", *Journal of Organic Chemistry*, Vol. 28, pp. 1339-1342, 1963; (5) Shih-Hsi Chu et al., "Potential Antiradiation Agents. II. Selenium Analogs of 2-Aminoethylisothiouronium Hydrobromide and Related Compounds", *Journal of the American Chemical Society*, Vol. 27, pp. 2899-2901, August, 1962; (6) Tohru Hino et al., "Radiation-protective Agents. I. Studies on N-Alkylated-2-(2-aminoethyl)thiopseudoureas and 1,1-(Dithioethylene)diguanidines", *Chemical & Pharmaceutical Bulletin*, Vol. 14, No. 11, pp. 1193-1201, November, 1966.

Suitable guanidino derivatives also are made according to the examples provided at the end of this detailed description of the invention.

In addition, suitable guanidino derivatives are made as indicated below:

General methods for the preparation of guanidines involve the reaction of amines with isoureas, isothioureas, sulphinic or sulphonic acid derivatives of thioureas, or with other guanylating agents such as cyanamides and pyrazole-N-carboxamidines. Relevant examples are found in Bannard et al., *Can. J. Chem.,* 36, 1541-1549 (1958); Walton, United Kingdom Patent No. 1 084 461 (1964); Bernatowicz et al., *J. Org. Chem.*, 57, 2497-2502 (1992); Maryanoff et al., *J. Org. Chem.*, 51, 1882-1884 (1986). Further relevant examples are referenced in Yamamoto & Kojima in "The Chemistry of Amidines and Imidates", J. Wiley & Sons (1991), pp. 485-526.

Hydroxyguanidine is prepared by the method of Walker & Walker, *J. Biol. Chem.*, 234, 1481-1484 (1959).

1-substituted hydroxyguanidines are prepared by the reaction of hydroxylamine with cyanamides or 1-substituted isothioureas. Relevant examples are described in: Fukuto et al., *Biochem Pharmacol.*, 43, 607-613 (1992); Sennequier et al., *Tet. Lett.*, 36, 6059-6062 (1995); Yoo & Fukuto, *Biochem. Pharm.*, 50,

1995-2000 (1995); Aurich & Scharpenberg, *Chem. Ber.*, 106, 1881-1896, (1973); Butler et al., The Biology of Nitric Oxide, Part 5, Portland Press, London, (1996), p. 179.

1,1-disubstituted hydroxyguanidines are prepared by the reaction of hydroxylamine with cyanamides or 1,1-disubstituted isothioureas. Relevent examples are found in: Fukuto et al., *Biochem. Pharmacol.*, 43, 607-613 (1992); Belzeki et al., *Bulletin De L'acadamie Polonaise Des Sciences, Serie de sciences chimiques xviii* (7), 375-378, (1970).

1,3-substituted hydroxyguanidines are prepared by the reaction of hydroxylamine with carbodiimides or N-substituted isothioureas. Relevent examples are found in: Miller et al., *Synth. Comm.*, 20, 217-226 (1990); Belzecki et al., *Bulletin De L'acadamie Polonaise Des Sciences, Serie de sciences chimiques xx*, 6, 499-503, (1972).

Higher substituted hydroxyguanidines are prepared by the reaction of hydroxylamine or N-substituted derivatives thereof with appropriately substituted isothioureas, carbodiimides or C-chloroformamidinium chlorides. Relevent examples are contained in: Vob et al., *Z. Chem.*, 13, 58 (1973); Ziman, *J. Org. Chem.*, 41, 3258 (1976); Gross et al., *J. F. Prakt. Chemie.*, 316, 434-442 (1974); Gross et al., *Ger. Offen.* #2040628 (1972); also U. S. Patent No. 4,000,196.

Aminoguanidines are prepared, for example, by the reaction of amines with S-methyl or S-ethyl-isothiosemicarbazide or N-substituted derivative thereof. Relevent examples are found in: Lieber & Smith, *Chem. Rev.*, 25, 213-277 (1939), (and references therein); Ruetten et al., *Br. J. Pharmacol.*, 117, (1996), (in press); European Patent Application No. 89 107 168.0 (filed April 20, 1989); U. S. Patent No. 3,972,932; U. S. Patent No. 210,291.

In addition, aminoguanidines are prepared by the reaction of hydrazines, or substituted derivatives thereof, with S-alkylisothioureas or N-substituted derivatives thereof. Relevent examples are found in : Lieber & Smith, *Chem. Rev.*, 25, 213-277 (1939), (and references therein); Kirsten & Smith, *J. Am. Chem. Soc.*, 58, 800-802 (1936).

Aminohydroxyguanidines are prepared by the reaction of hydroxylamine or an N-substituted derivative thereof with S-methyl or S-ethyl- isothiosemicarbazide or N-substituted derivatives thereof. Relevent examples are found in: Houlihan et al., U. S. Patent No. 3,927,096; Tai et al., *J. Med. Chem.*, 27, 236-238 (1984).

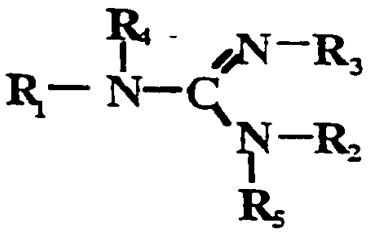
Mercaptoalkylguanidines and their S-substituted derivatives are prepared by reacting appropriate mercaptoalkylamine or S-substituted mercaptoalkylamine with suitable guanylating reagent. Relevent examples are found in: Southan et al., *Br. J. Pharmacol.*, 117, 619-633 (1996).

Mercaptoethyl- and mercaptopropyl-guanidine, and their N-substituted derivatives, are prepared by neutralizing a solution of the appropriate N^G-substituted aminoethyl- or aminopropyl-isothiourea to induce rearrangement of the latter into the former. Said N^G-substituted aminoethyl- or aminopropyl-isothioureas are prepared by alkylation of thiourea with aminoalkylhalides, preferably the bromide. Relevent examples are found in: Khym et al., *J. Am. Chem. Soc.*, 79, 5663-5666 (1957); Doherty & Shapira, *J. Org. Chem.*, 28, 1339-1342 (1963); Khym et al., *J. Am. Chem. Soc.*, 80, 342-3349 (1958); Doherty et al., *J. Am. Chem. Soc.*, 79, 5667-5671 (1957); Hino et al., *Chem. & Pharm. Bull.*, 14, 1193-1201 (1966); Shapira et al., *Rad. Res.*, 7, 22-34 (1957); DiStephano et al., *Rad. Res.*, 18, 177-185 (1963); Southan et al., *Br. J. Pharmacol.*, 117, 619-633 (1996).

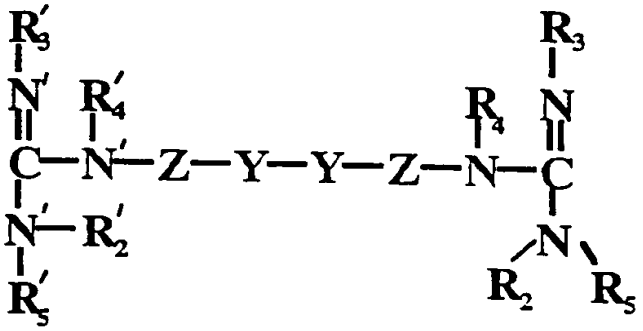
Selenoethyl- or selenopropylguanidine or their N-substituted derivatives are prepared by neutralizing a solution of the appropriate N^G-substituted aminoethyl- or aminopropyl-isoselenourea to induce rearrangement of latter into the former. Said N^G-substituted aminoethyl- or aminopropyl-isoselenoureas are prepared by alkylation of selenourea with aminoalkylhalides, preferably the bromide. Relevent examples are found in: Chu & Mautner, *J. Org. Chem.*, 27, 2899-2901 (1962); Southan et al., *Life Sci.*, 58, 1139-1148 (1996).

Heterocyclic guanidine derivatives are prepared as shown in: Schantl & Turk, *Sci. Pharm.* (scientia pharmaceutica), 57, 375-380 (1989); Fukuto et al., *Biochem. Pharmacol.*, 43, 607-613 (1992); Belzecki et al., *Bulletin De L'acadamie Polonaise, Serie de sciences chimiques xviii* (7), 375-378 (1970); Belzecki & Trojnar, *Bulletin De L'acadamie Polonaise Des Sciences, Serie de sciences chimiques xviii* (8), 437-440 (1970); Belzecki & Trojnar, *Tet. Lett.*, 22, 1879-1880 (1970).

The guanidino derivative of the composition and method is defined by a formula selected from the group consisting of:



and



or a salt thereof, wherein:

R_2 and R'_2 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, aminoalkyl, hydroxy, alkoxy,

thioalkylene, thioesteralkylene, phenyl or phenylalkylene, or a substituted derivative thereof;

R_3 and R'_3 are independently H, lower alkyl, alkylene, alkenylene, amino, hydroxy, thioalkylene, or a substituted derivative thereof;

R_1, R_5, R'_5, R_4 and R'_4 are independently H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene or amino, or a substituted derivative thereof;

Alternatively, R_1 is R_6-Y-Z- where R_6 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene, acyl, $-SO_3^-$, or $-PO_3^-$, or a substituted derivative thereof, and Z and Y are as defined below;

Z and Z' are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

Y and Y' are independently S or Se;

When R_2 or R'_2 is alkylene, alkenylene, thioalkylene, amino, hydroxy or a substituted derivative thereof, said R_2 or R'_2 may be joined to any of:

(i) R_3 or R'_3, if R_3 or R'_3 is alkylene, alkenylene or thioalkylene;

(ii) R_4 or R'_4, if R_4 or R'_4 is alkylene or alkenylene; or

(iii) R_5 or R'_5, if R_5 or R'_5 is alkylene or alkenylene;

to form 5-, 6-, or 7-membered heterocycle;

When R_2, R_3, R'_2 or R'_3 is alkylene or alkenylene, said R_2, R_3, R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6-membered heterocyclic ring, with the proviso that said heterocyclic ring optionally being substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

When R_1 and R_4 are alkylene or alkenylene, said R_1 and R_4 optionally may be joined together to form a 5-, 6-, or 7-membered heterocycle;

When R_1 is R_6-Y-Z-, and R_6 is alkylene or alkenylene, R_6 optionally may be joined to any of:

(i) R_2, when R_2 is alkylene, alkenylene or thioalkylene;

(ii) Z; or

(iii) R_4, when R_4 is alkylene or alkenylene;

to form a 5-, 6- or 7-membered heterocyclic ring; and

a pharmaceutically acceptable carrier, said compound present in said composition in an effective amount to inhibit the cytotoxic effect of peroxynitrite in said mammal.

As used herein, the term "salt" refers to any addition salt derived from any pharmaceutically acceptable organic or inorganic acid. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene p sulfonic, tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-

sulfonic and benzenesulphonic acids. Additionally, as used herein, any alkyl or alkylene may be straight chain, branched or cyclic, and "halo" includes bromine, chlorine, fluorine and iodine.

In the descriptions mentioned above, a substituted derivative of R_2, R_3, R'_2 and R'_3 refers to a derivative that may be substituted with one or more alkoxy, halo, hydroxy and amino groups. A substituted derivative of R_1, R_5, R'_5, R_4 and R'_4 refers to a derivative that may be substituted with one or more alkyl, alkoxy, halo, hydroxy, amino, amino alkyl (secondary or tertiary), thio and nitro groups.

If the R_2, R'_2, R_3 or R'_3 substituent is thioalkylene, the thioalkylene preferably has a formula $[-(CH_2)_n-SH]$ where n is independently 1 to 4. If the R_2, or R'_2 substituent is thioesteralkylene, the thioesteralkylene preferably has the formula $[-(CH_2)_n-S-R_7]$ where R_7 is independently a lower alkyl and n is independently 1 to 4.

The Z and Z' substituents of the guanidino derivative are independently alkylene, alkenylene, cycloalkyene or cycloalkenlyene, or a substituted derivative thereof. When such a substituted derivative is employed, the substituent may include one or more of lower alkyl, alkoxy, halo, amino, nitro or carboxyl.

A preferred subgroup of derivatives includes compounds where: R_2 and R'_2 are independently H, lower alkyl, amino,

aminoalkyl, hydroxy, phenyl, phenylalkylene, or a substituted derivative thereof; R_3 and R'_3 are independently H, lower alkyl, amino or hydroxy; R_5 and R'_5 , R_4 and R'_4 are independently H or lower alkyl; R_1 is H, lower alkyl or R_6-Y-Z- where R_6 is H, lower alkyl, acyl, $-SO_3^-$, $-PO_3^-$, or a substituted derivative thereof; Z and Z' are independently alkylene, optionally substituted with one or more substituents chosen from lower alkyl or carboxylic acid; and Y and Y' are S.

Another preferred subgroup includes guanidino derivatives where: R_1 is H or alkyl; R_2 is H, amino, hydroxy, methoxy or ethoxy; R_3 is H, lower alkyl or amino; R_4 is H; and R_5 is H or lower alkyl. A few nonlimiting examples include aminoguanidine, hydroxyguanidine, 1-amino-2-hydroxyguanidine, 1-amino-2-methyl-2-hydroxyguanidine and diaminoguanidine.

Yet another preferred subgroup includes mercapto derivatives where: R_1 is R_6-Y-Z- where R_6 is H, acyl, $-SO_3^-$, $-PO_3^-$ or lower alkyl, R_2 and R'_2 are independently H or amino; R_3 and R'_3 are independently H, amino or hydroxy; R_4 and R'_4 are independently H, methyl or ethyl; Z and Z' are independently alkylene optionally substituted with one or more methyl; and Y is S. A few nonlimiting examples include mercaptoethylguanidine, mercaptopropylguanidine, S-methylpropylguanidine, mercaptoethylguanidine-S-phosphoric acid (S-(guanidinoethyl) phosphorothioic acid) and guanidinoethyldisulfide.

The guanidino derivative, in pure form or in a pharmaceutically acceptable carrier, will find benefit in treating conditions and disorders where there is an advantage in inhibiting the cytotoxic effect of peroxynitrite. For example, the guanidino derivative may be used to treat a circulatory shock including its various aspects such as vascular and myocardial dysfunction, metabolic failure including the inhibition of mitochondrial enzymes and cytochrome P450-mediated drug metabolism, and multiple organ dysfunction syndrome including adult respiratory distress syndrome. Circulatory shock may be a result of gram-negative and gram positive sepsis, trauma, hemorrhage, burn injury, anaphylaxis, cytokine immunotherapy, liver failure, kidney failure or systemic inflammatory response syndrome. Guanidino derivatives also may be beneficial for patients receiving therapy with cytokines such as TNF, IL-1 and IL-2 or therapy with cytokine-inducing agents, or as an adjuvant to short term immunosuppression in transplant therapy.

Peroxynitrite is formed and is cytotoxic in the reperfusion phase of various forms of ischemia-reperfusion injury. There is, therefore, a distinct advantage in inhibiting the cytotoxic effect of peroxynitrite in these conditions. The pathophysiological conditions associated with ischemia-reperfusion injury include myocardial ischemia and infarction, cardiopulmonary bypass, noninflammatory diseases of the central nervous system (CNS)

including stroke (a CNS ischemic disorder) and cerebral ischemia, and hemorrhagic shock.

There is also evidence that peroxynitrite may be involved in the pathophysiology of autoimmune and/or inflammatory conditions such as arthritis, rheumatoid arthritis, inflammatory bowel disease and myocarditis, systemic lupus erythematosus (SLE) and insulin-dependent diabetes mellitus, and therefore, guanidino derivatives may prove helpful in treating these conditions.

Furthermore, it is now clear that there are a number of additional inflammatory and noninflammatory diseases and conditions that may be associated with peroxynitrite production. Examples of such physiological disorders include: inflammatory bowel diseases such as ileitis and Crohn's disease; inflammatory lung disorders such as asthma and chronic obstructive airway disease; inflammatory disorders of the eye including corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis and endophthalmitis; chronic inflammatory disorders of the gum including periodontitis; chronic inflammatory disorders of the joints including arthritis and osteoarthritis, tuberculosis, leprosy, glomerulonephritis sarcoid, and nephrosis; disorders of the skin including sclerodermatitis, psoriasis and eczema; inflammatory diseases of the central nervous system, including chronic demyelinating diseases such as multiple sclerosis, dementia including AIDS-related

neurodegeneration and Alzheimer's disease, encephalomyelitis and viral or autoimmune encephalitis; autoimmune diseases including immune-complex vasculitis, systemic lupus and erythematodes; and disease of the heart including ischemic heart disease and cardiomyopathy. Additional diseases and conditions which may benefit from the use of the guanidino derivative include adrenal insufficiency; hypercholesterolemia; atherosclerosis; bone disease associated with increased bone resorption, e.g., osteoporosis, pre-eclampsia, eclampsia, uremic complications, chronic liver failure, various forms of cancer, Parkinson's disease, spinal cord trauma and head trauma.

In addition, the guanidino derivative may be used as a preservative solution, or as an additive to a preservative solution, for use in preserving a harvested organ prior to transplantation.

Pharmaceutical formulations of the guanidino derivative may include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All such pharmacy methods include the steps of bringing into association the active compound with liquid carriers or

finely divided solid carriers or both as needed and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented: as discrete units, such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; or as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus electuary or paste, and be in a pure form, i.e., without a carrier. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such

liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include: aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges,

comprising the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs.

For administration by inhalation the compounds according to the invention are conveniently delivered from an insufflator, nebulizer pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for

example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

When desired the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions according to the invention may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

The compounds of the invention may also be used in combination with other therapeutic agents, for example, anti-inflammatory agents, particularly nitric oxide synthase inhibitors, cyclooxygenase blockers, superoxide scavengers, vasodilator prostaglandins including prostacyclin and prostaglandin E_1, cancer chemotherapeutic agents including cisplatin, NO donors or NO inhalation therapy, or PAF-receptor antagonists.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the guanidino derivative may be administered orally or via injection at a dose of from 0.1 to 250 mg/kg per day. The dose range for adult humans is generally from 5 mg to 17.5 g/day, preferably 5 mg to 10 g/day and most preferably 100 mg to 3 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 100 mg to 500 mg.

The pharmaceutical composition preferably is administered orally or by injection (intravenous or subcutaneous), and the precise amount administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend upon a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

While not intending to be bound by any particular theory, or to limit the scope of the invention to any such theory, we postulate that the salutory effect of the guanidino derivatives of the present invention is due, at least in large part, to a reaction between the guanidino derivatives and peroxynitrite, thereby converting peroxynitrite to less toxic, less biologically active species, including

nitrite ($NO_2^{\cdot}$). In this manner, the guanidino compounds are acting as scavengers of peroxynitrite, facilitating the rapid decomposition of peroxynitrite to less biologically active species. We further believe that this scavenging is happening in a manner, and at a rate, that is competitive with the reaction of peroxynitrite with endogenous biomolecules, so as to reduce or prevent: (i) the occurrence of species otherwise produced by the reaction of peroxynitrite with such endogenous biomolecules, or (ii) the inappropriately altered function of such biomolecules.

It is envisaged that decomposition or reaction of peroxynitrite can proceed either via transfer of one electron to peroxynitrite or via direct nucleophilic attack of peroxynitrite by the scavenger via an S_N2 type mechanism.

For example, the reaction of S-methyl mercaptoethylguanidine with peroxynitrite may involve one electron transfer:

(a) $G\sim S\cdot CH_3 + HOONO \longrightarrow G\sim \overset{+\cdot}{S}\cdot CH_3 + OH^- + \dot{N}O_2$

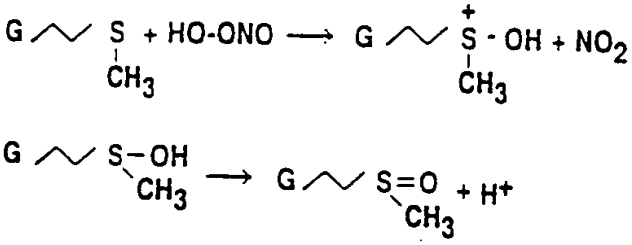
where G represents the guanidino group ($H_2NC(=NH)NH$-).

Within the solvent cage, further reaction can occur to give nitrite and the sulphoxide derivative of S-methyl mercaptoethylguanidine:

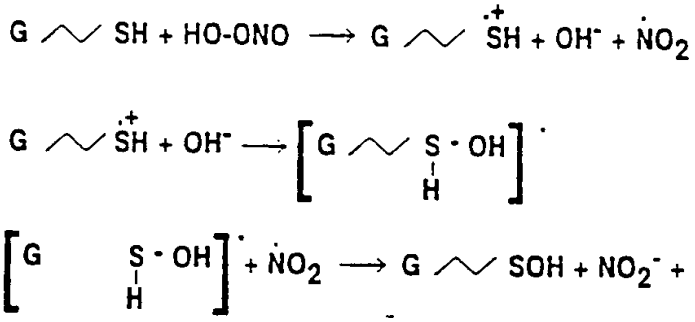
(b) $G\sim \overset{+\cdot}{S}\cdot CH_3 + OH^- \longrightarrow \left[G\sim \underset{\underset{O\text{-}H}{|}}{S}\cdot CH_3\right]^{\cdot}$

$$\left[G\sim \underset{\underset{OH}{|}}{S}\cdot CH_3\right]^{\cdot} + \dot{N}O_2 \longrightarrow G\sim \underset{\underset{O}{||}}{S}\cdot CH_3 + NO_2^- + H^+$$

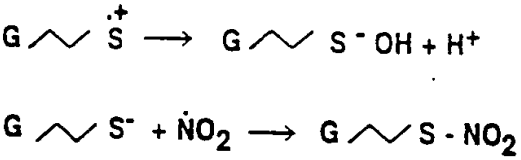
The same products can be derived from an S_N2 type mechanism:

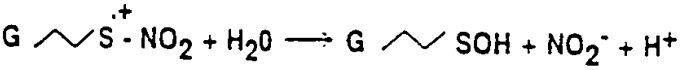
(c) 

Similar reaction schemes can be drawn for the reaction of mercaptoethylguanidine with peroxynitrite:

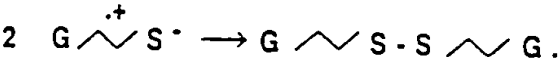
(d) 

However, an alternative pathway can result in the same sulphenic acid and nitrite products:

(e) 

(f) 

In addition, the disulphide can result:

(g) 

Again, an S_N2 type mechanism can lead to the same products:

(h) $G \sim SH + HOONO \longrightarrow G \sim \overset{+}{\underset{H}{S}}-OH + NO_2^-$

$G \sim \overset{+}{\underset{H}{S}}-OH \longrightarrow G \sim SOH + H^+$

(OR)

(i) $G \sim SH + ONOOH \longrightarrow G \sim S-NO_2 + H_2O$

$G \sim S-NO_2 + H_2O \longrightarrow G \sim SOH + NO_2^- + H^+$

In line with current theory regarding peroxynitrite, the peroxynitrite species illustrated above as HOONO may be regarded as an activated form (see Pryor and Squadrito: W. A. Pryor & G. L. Squadrito, "The chemistry of peroxynitrite", *Am. J. Physiology*, 268, 699-722 (1995)).

Although reaction schemes are drawn for neutral reactants, it is also possible, and even probable, that the peroxynitrite anion ($ONOO^-$) or, for the examples illustrated, the thiolate anion (RS^-) may be the participating species. It is further possible that intermediates other than those illustrated above are formed. To illustrate these points, the following may be hypothesized:

(j) $G \sim S^- + HOONO \longrightarrow G \sim S-ONO$

$G \sim S-ONO \longrightarrow G \sim S^{\cdot} + {}^{\cdot}NO_2$

$2\,G \sim S^{\cdot} \longrightarrow G \sim S-S \sim G$

Direct reaction products may be further metabolized/oxidized to give other products. For example, sulphenic acid derivatives may be oxidized to the corresponding sulphinic or sulphonic acid derivatives, or give rise to the disulphides.

The above pathways illustrate possible mechanisms for the decomposition of peroxynitrite to nitrite by sulphur-containing scavengers. Other scavengers, or these scavengers, may act by similar, unrelated or multiple mechanisms, depending on the nature of the molecule as a whole. The above reaction pathways are intended to demonstrate that the guanidino compounds of the invention may operate by causing the decomposition of peroxynitrite to less reactive toxic species such as nitrite. However, the invention is not limited to these postulated mechanisms.

The following Examples are provided by way of illustration, and are not intended to limit the scope of the invention.

The guanidino derivatives used in Examples 1-4 were prepared as follows: mercaptoethylguanidine (MEG) was prepared as shown in Example 5, guanidino ethyldisulphide (GED) was made according to Example 6, S-methyl mercaptoethylguanidine (SMEG) was prepared according to the method of Example 7, and aminoguanidine (AG) was purchased from Aldrich Chemical Co., Milwaukee, WI, in the form of amoniguanidinehydrochloride.

EXAMPLE 1

With reference to the results shown in Fig. 1, this example illustrates the effect of mercaptoethylguanidine (MEG), S-methyl-mercaptoethylguanidine (SMEG), guanidinoethyldisulfide (GED) and aminoguanidine (AG) on the *in vitro* oxidation of dihydrorhodamine 123 (DHR) by peroxynitrite (n = 3-6). These studies were performed in phosphate-buffered saline containing 50 μM dihydrorhodamine 123 at pH 7.4. The oxidation of dihydrorhodamine 123 was induced by rapid mixing with peroxynitrite (3.3 μM final concentration) in the presence of various concentrations of the guanidino compounds. After 10 min, the amount of rhodamine 123 formed (the oxidation product of dihydrorhodamine 123) was measured using a fluorescent spectrophotometer (excitation: 500 nm, emission: 536 nm).

Legend to Fig. 1. Effect of mercaptoethylguanidine (MEG), S-methyl-mercaptoethylguanidine (SMEG), guanidinoethyldisulfide (GED) and aminoguanidine (AG) on the oxidation of dihydrorhodamine 123 (DHR) in response to peroxynitrite (PN).

EXAMPLE 2

With reference to the results shown in Figs. 2A-2C, this example illustrates the effect of aminoguanidine (AG), mercaptoethylguanidine (MEG), and guanidinoethyldisulfide (GED) on

the decrease in mitochondrial respiration in J774 macrophages exposed to peroxynitrite (N = 3-6). J774 macrophage cell lines were obtained from the American Type Culture Collection (ATCC) and were grown using standard methods in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, glutamine, penicillin (10,000 U/l) and streptomycin (10,000 U/l). Cells were grown in 96-well plates for measurement of mitochondrial respiration and in 12-well plates for measurement of DNA strand breakage (DNA strand breakage is discussed in Example 3, below). All the experiments were carried out with 10% fetal calf serum.

Cells were pretreated with the indicated doses of the guanidino compounds for 10 minutes. Then, cells were exposed to peroxynitrite (1 mM final concentration). After 1 h, mitochondrial respiration was measured. C represents a control culture, which received no pretreatment with a guanidino compound and which was not exposed to peroxynitrite.

Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of MTT [3-(4,5 - dimethylthiazol-2-yl) - 2,5 - diphenyltetrazolium bromide] to formazan. Cells in 96-well plates were incubated (37°C) with MTT (0.2 mg/ml for 60 minutes). Culture medium was removed by aspiration and the cells solubilized in dimethylsulfoxide (DMSO) (100 μl). The extent of reduction of MTT to formazan within cells was

quantitated by measurement of OD_{550} using a microplate reader. The calibration curve for the reduction of MTT to formazan was prepared in DMSO. Formazan production by cells was expressed as a percentage of the values obtained from untreated cells.

Cells exposed to peroxynitrite showed a markedly suppressed mitochondrial respiration at 1 h. This suppressed respiration was dose-dependently inhibited by the guanidino derivatives, with mercaptoethylguanidine (MEG) being the most potent inhibitor (see Figs. 2A-2C).

Legend to Fig. 2. Effect of aminoguanidine (AG), mercaptoethylguanidine (MEG) and guanidinoethyldisulfide (GED) on the suppression of mitochondrial respiration in response to peroxynitrite (PN).

EXAMPLE 3

With reference to the results shown in Fig. 3, this example illustrates the effect of aminoguanidine (AG), mercaptoethylguanidine (MEG), and guanidinoethyldisulfide (GED) (100 μM each) on the increase in DNA single strand breaks in J774 macrophages exposed to peroxynitrite (PN) (1 mM) (N = 3-6). J774 macrophage cell lines were obtained and cultured as described in Example 2. Macrophages were then exposed to peroxynitrite for 30 min. Then, the percentage of DNA single strand breaks was

determined by the alkaline unwinding method. C represents a control culture, which received no pretreatment with a guanidino compound and which was not exposed to peroxynitrite. At the end of the incubation period, cells were scraped into 0.2 ml of solution A buffer (myoinositol 250mM, NaH_2PO_3 10 mM, $MgCl_2$ 1 mM, pH 7.2). The cell lysate was then transferred into plastic tubes designated T (maximum fluorescence), P (fluorescence in sample used to estimate extent of DNA unwinding), or B (background fluorescence). To each tube, 0.2 ml of solution B (alkaline lysis solution: NaOH 10 mM, urea 9 M, ethylenediaminetetraacetic acid 2.5 mM, sodium dodecyl sulfate 0.1%) was added and incubated at 4 °C for 10 minutes to allow cell lysis and chromatin disruption. 0.1 ml each of solutions C (0.45 volume solution B in 0.2 N NaOH) and D (0.4 volume solution B in 0.2 N NaOH) was then added to the P and B tubes. 0.1 ml of solution E (neutralizing solution: glucose 1 M, mercaptoethanol 14 mM) was added to the T tubes before solutions C and D were added. From this point incubations were carried out in the dark. A 30-minute incubation period at 0 °C was then allowed during which the alkali diffused into the viscous lysate. Since the neutralizing solution, solution E, was added to the T tubes before addition of the alkaline solutions C and D, the DNA in the T tubes was never exposed to a denaturing pH. At the end of the 30 minute incubation, the contents of the B tubes were sonicated for 30 seconds to ensure

rapid denaturation of DNA in the alkaline solution. All tubes were then incubated at 15 °C for 10 minutes. Denaturation was stopped by chilling to 0 °C and adding 0.4 ml of solution E to the P and B tubes. 1.5 ml of solution F (ethidium bromide 6.7 μg/ml in 13.3 mM NaOH) was added to all the tubes and fluorescence (excitation: 520 nm, emission: 590 nm) was measured spectrophometrically. Under the conditions used, in which ethidium bromide binds preferentially to double stranded DNA, the percentage of double stranded DNA (D) may be determined using the equation: % D = 100 X [F(P) - F(B)]/[F(T) - F (B)]; where F(P) is the fluorescence of the sample, F(B) the background fluorescence, i.e. fluorescence due to all cell components other than double stranded DNA, and F(T) the maximum fluorescence.

Fig. 3 shows that pretreatment of the cells with aminoguanidine (AG), mercaptoethylguanidine (MEG) and guanidinoethyldisulfide (GED) (100 μM each) protected against the peroxynitrite (PN) (1 mM)-induced single strand breakage.

Legend to Fig. 3. Percentage of DNA single strand breaks in control (C, untreated macrophages), in cells exposed to peroxynitrite (PN), and in cells exposed to peroxynitrite in the presence of aminoguanidine (AG), mercaptoethylguanidine (MEG) or guanidinoethyldisulfide (GED) (n = 6).

EXAMPLE 4

With reference to the results shown in Fig. 4, this example illustrates the effect of mercaptoethylguanidine (MEG) on the peroxynitrite-induced suppression of contractility in vascular rings. Thoracic aortae from rats were cleared of adhering periadventitial fat and cut into rings of 1-4 mm width. Rings in Krebs' solution were exposed to peroxynitrite (ONOO) (300 μM) or vehicle control, in the presence or absence of 100 μM MEG> following a 30 minute incubation, rings were set up for the measurements of isometric contractility. The rings were mounted in organ baths (5 ml) filled with warmed (37 °C) oxygenated (95% O_2/5%CO_2 Krebs' solution (pH 7.4) consisting of (mmol/L): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.7, in the presence of indomethacin (10 μmol/L). Isometric force was measured with isometric transducers (Kent Scientific Corp. Litchfield, CT, USA), digitalized using a Maclab A/D converter (AD Instruments, Milford, MA, USA), and stored and displayed on a Macintosh personal computer. A tension of 1 g was applied and the rings were equilibrated for 60 min. Fresh Krebs' solution was provided at 15 minute intervals. After the incubation period, concentration-response curves to noradrenaline (10^{-9}-10^{-5} mol/L) were obtained.

Peroxynitrite induced a marked suppression of vascular contractility. However, MEG caused a partial protection against the peroxynitrite-induced suppression of vascular contractility.

Legend to Figure 4. Concentration-response curves to noradrenaline for control vascular rings, for rings exposed to peroxynitrite (PN), and for rings exposed to peroxynitrite (PN) in the presence of mercaptoethylguanidine (MEG).

EXAMPLE 5

This example illustrates a method for synthesizing mercaptoethylguanidine sulfate. Mercaptoethylamine hydrochloride (2g) was dissolved in methanol (5 ml) and cooled in a salt/ice bath. A cold solution of potassium hydroxide (0.99 g) in methanol (10 ml) was added and the mixture stirred. After 1 hour, the solution was filtered and S-methylisothiourea (2g) was added to 12 ml of the filtrate. The solution was stirred at room temperature (18°C) for 16 hours under nitrogen. The solution was then filtered and ether was added to precipitate the crude product which was then recrystallized from an ether/ethanol mixture.

EXAMPLE 6

A further example is for the preparation of guanidinoethyldisulphide (GED) dihydrochloride as follows: to a solution of cystamine dihydrochloride (.5 g, 2.2 mmol) in water (25

ml) was added 10 ml of Amberlite IRA 402 (OH) resin, followed by 1H-pyrozole-1-carboxamide HCl (0.98 g, 6.6 mmol). The mixture was stirred for 16 h at room temperature, the resin removed and the filtrate extracted with ethylacetate. The aqueous layer was acidified with HCl to pH2 and lyophilised to afford 0.56 g GED dihydrochloride.

EXAMPLE 7

2-(Methylthio)ethylguanidine sulphate was prepared as follows: to a solution of 0.695 g S-methylisothiourea in 15 ml of 90% methanol was added 0.456 g 2-(methylthio)ethylamine. The solution was stirred for 20 h at room temperature, filtered and the solvent removed *in vacuo*. The residue was crystallized from a mixture of methanol and ether.

EXAMPLE 8

2-(ethylthio)ethylguanidine sulphate was prepared using the procedure of example 5; however, 0.5 g of 2-(ethylthio)ethylamine was used instead of 2-(methylthio)ethylamine.

EXAMPLE 9

N-amidinylthiomorpholine sulphate was prepared as follows: thiomorpholine (3 ml) was added to a solution of 4.17 g S-methylisothiourea in 30 ml of 25% aqueous methanol and the solution was stirred overnight. The solvent was removed under reduced pressure and the residue taken up in warm methanol and

filtered. The volume was reduced and the solution was left for 2 days after which the solid was collected.

EXAMPLE 10

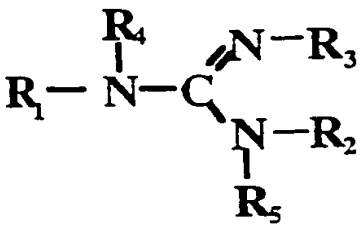
N-amidinylthiazolidine sulphate was prepared as follows: thiazolidine (1 g) was added to a solution of 1.56 g S-methylisothiourea in 15 ml of 25% aqueous methanol and the solution was stirred overnight. The solvent was removed under reduced pressure and the residue recrystallized from methanol/water to give a white solid in low yield.

The detailed description of the invention presented above is provided by way of illustration, and it is not intended to limit the scope of the invention which is to be determined by the following claims.

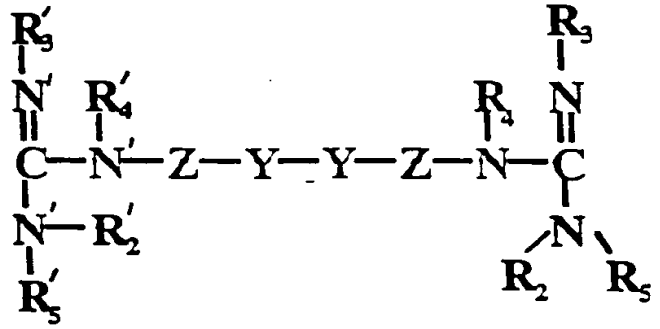
WHAT IS CLAIMED IS:

1. A pharmacologically acceptable composition for inhibiting the cytotoxic effect of peroxynitrite in a mammal, comprising:

a compound having a formula selected from the group consisting of:



and



or a salt thereof, wherein:

R_2 and R'_2 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, aminoalkyl, hydroxy, alkoxy, thioalkylene, thioesteralkylene, phenyl or phenylalkylene, or a substituted derivative thereof;

R_3 and R'_3 are independently H, lower alkyl, alkylene, alkenylene, amino, hydroxy, thioalkylene, or a substituted derivative thereof;

R_1, R_5, R'_5, R_4 and R'_4 are independently H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene or amino, or a substituted derivative thereof;

Alternatively, R_1 is R_6-Y-Z- where R_6 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene, acyl, $-SO_3^{--}$, or $-PO_3^{--}$, or a substituted derivative thereof, and Z and Y are as defined below;

Z and Z' are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

Y and Y' are independently S or Se;

When R_2 or R'_2 is alkylene, alkenylene, thioalkylene, amino, hydroxy or a substituted derivative thereof, said R_2 or R'_2 may be joined to any of:

(i) R_3 or R'_3, if R_3 or R'_3 is alkylene, alkenylene or thioalkylene;

(ii) R_4 or R'_4, if R_4 or R'_4 is alkylene or alkenylene; or

(iii) R_5 or R'_5, if R_5 or R'_5 is alkylene or alkenylene;

to form 5-, 6-, or 7-membered heterocycle;

When R_2, R_3, R'_2 or R'_3 is alkylene or alkenylene, said R_2, R_3, R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6-membered heterocyclic ring, with the proviso that said heterocyclic ring optionally is substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

When R_1 and R_4 are alkylene or alkenylene, said R_1 and R_4 optionally may be joined together to form a 5-, 6-, or 7-membered heterocycle;

When R_1 is R_6-Y-Z-, and R_6 is alkylene or alkenylene, R_6 optionally may be joined to any of:

(i) R_2, when R_2 is alkylene, alkenylene or thioalkylene;

(ii) Z; or

(iii) R_4, when R_4 is alkylene or alkenylene;

to form a 5-, 6- or 7-membered heterocyclic ring; and

a pharmaceutically acceptable carrier, said compound present in said composition in an effective amount to inhibit the cytotoxic effect of peroxynitrite in said mammal.

2. The composition of claim 1 wherein said substituted derivative of R_2, R_3, R_2 or R'_3 is independently selected from the group consisting of one or more of alkoxy, halo, hydroxy and amino.

3. The composition of claim 1 wherein said substituted derivative of R_1, R_5, R'_5, R_4 or R'_4 is independently selected from the group consisting of one or more of alkyl, alkoxy, halo, hydroxy, amino, amino alkyl (secondary or tertiary), thio and nitro.

4. The composition of claim 1 wherein said R_2, R_3, R'_2 or R'_3 thioalkylene has a formula $[-(CH_2)_n-SH]$ where n is independently 1 to 4.

5. The composition of claim 1 wherein said R_2 or R'_2 thioesteralkylene has a formula $[-(CH_2)_n-S-R_7]$ where R_7 is independently a lower alkyl and n is independently 1 to 4.

6. The composition of claim 1 wherein said substituted derivative of Z or Z' is independently selected from the group consisting of one or more of lower alkyl, alkoxy, halo, amino, nitro and carboxyl.

7. The composition of claim 1 wherein:

R_2 and R'_2 are independently selected from the group consisting of H, lower alkyl, amino, aminoalkyl, hydroxy, phenyl, phenylalkylene and a substituted derivative thereof;

R_3 and R'_3 are independently selected from the group consisting of H, lower alkyl, amino and hydroxy;

R_5, R'_5, R_4 and R'_4 are independently selected from the group consisting of H and lower alkyl;

R_1 is selected from the group consisting of H, lower alkyl and R_6-Y-Z-, where R_6 is selected from the group consisting of H, lower alkyl, acyl, $-SO_3^-$, $-PO_3^-$ and a substituted derivative thereof;

Z and Z' are independently alkylene, optionally substituted with one or more substituents selected from the group consisting of lower alkyl and carboxyl; and

Y and Y' are S.

8. The composition of claim 1 wherein:

R_1 is selected from the group consisting of H and alkyl;

R_2 is selected from the group consisting of H, amino, hydroxy, methoxy and ethoxy;

R_3 is selected from the group consisting of H, lower alkyl and amino;

R_4 is H; and

R_5 is selected from the group consisting of H and lower alkyl.

9. The composition of claim 1 wherein said compound is selected from the group consisting of aminoguanidine, hydroxyguanidine, 1-amino-2-hydroxyguanidine, 1-amino-2-methyl-2-hydroxyguanidine and diaminoguanidine.

10. The composition of claim 1 wherein:

R_1 is R_6-Y-Z-;

R_6 is selected from the group consisting of H, acyl, $-SO_3^-$, $-PO_3^-$ and lower alkyl;

R_2 and R'_2 are independently selected from the group consisting of H and amino;

R_3 and R'_3 are independently H, amino and hydroxy;

R_4 and R'_4 are independently H, methyl and ethyl;

Z and Z' are independently alkylene optionally substituted with one or more methyl; and

Y is S.

11. The composition of claim 1 wherein said compound is selected from the group consisting of mercaptoethylguanidine, mercaptopropylguanidine, S-methyl-mercaptoethylguanidine, S-methyl-mercaptopropylguanidine, 2-mercapto-2-methylpropylguanidine, mercaptoethylguanidine-S-phosphoric acid and guanidinoethyldisulfide.

12. The composition of claim 1 wherein said compound reacts with the peroxynitrite, thereby inhibiting the cytotoxic effect of the peroxynitrite.

13. The composition of claim 1 for inhibiting peroxynitrite-induced oxidative reactions, thereby inhibiting the cytotoxic effect of the peroxynitrite.

14. The composition of claim 1 for inhibiting peroxynitrite-induced suppression of cellular respiration.

15. The composition of claim 1 for inhibiting peroxynitrite-induced DNA strand breakage.

16. The composition of claim 1 for inhibiting peroxynitrite-induced suppression of vascular smooth muscle contractility.

17. The composition of claim 1 wherein said compound is present in an amount sufficient to treat a condition where there is an advantage in inhibiting the cytotoxic effect of peroxynitrite.

18. The composition of claim 17 wherein said condition is selected from the group consisting of circulatory shock, systemic inflammatory response syndrome, therapy with cytokines, ischemia-reperfusion injury of the heart, ischemia-reperfusion injury of the brain, therapy with cytokine-inducing agents, transplantation, transplant rejection, local inflammatory responses, systemic inflammation, autoimmune diseases, adult respiratory distress syndrome, arthritis, rheumatoid arthritis, diabetes mellitus, ileitis, ulcerative colitis, Crohn's disease, asthma, periodontitis, nephrosis, chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related complications, Alzheimer's disease, cardiomyopathy, adrenal insufficiency, hypercholesterolemia, atherosclerosis, bone diseases associated with increased bone resorption, pre-eclampsia, eclampsia, uremic complications, chronic liver failure, stroke, and cancer.

19. The composition of claim 17 wherein said condition is selected from the group consisting of circulatory shock, myocardial ischemia and a central nervous system ischemic disorder.

20. The composition of claim 1 formulated for oral, rectal, nasal, topical, buccal, sub-lingual, vaginal, parenteral, intramuscular, sub-cutaneous, intravenous, inhalation or insufflation administration.

21. The composition of claim 1 formulated for oral administration, said carrier including an ingredient selected from the group consisting of a binding agent, filler, lubricant, disintegrant, wetting agent, inert diluent, surface active agent, dispersing agent, suspending agent, emulsifying agent, edible oil, flavoring agent and mixtures thereof.

22. The composition of claim 1 formulated for topical administration in the mouth, said carrier including an ingredient selected from the group consisting of a flavor, sucrose, acacia, tragacanth, gelatin, glycerin and mixtures thereof.

23. The composition of claim 1 formulated for nasal administration, said carrier including an ingredient selected from the group consisting of a dispersing agent, solubilizing agent, suspending agent and mixtures thereof.

24. The composition of claim 1 formulated for administration by inhalation, said carrier including a propellant.

25. The composition of claim 24 wherein said propellant is selected from the group consisting of dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide and mixtures thereof.

26. The composition of claim 1 formulated for administration by inhalation or insufflation, said carrier including an ingredient selected from the group consisting of lactose, starch and mixtures thereof.

27. The composition of claim 1 formulated for parenteral administration, said carrier including an ingredient selected from the group consisting of an anti-oxidant, buffer, bacteriostat, suspending agent, thickening agent, saline, water and mixtures thereof.

28. The composition of claim 1 formulated for rectal administration, said carrier including an ingredient selected from the group consisting of cocoa butter, polyethylene glycol and mixtures thereof.

29. The composition of claim 1 formulated to include an ingredient selected from the group consisting of an antimicrobial agent, an immunosuppressant, a preservative and mixtures thereof.

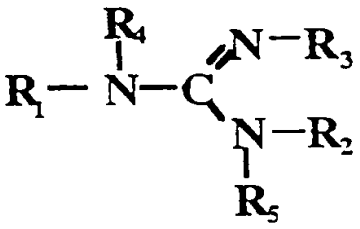
30. The composition of claim 1 formulated for administration at a dose of from about 5 mg to about 17.5 g/day of said compound.

31. The composition of claim 30 formulated for administration at a dose of from about 5 mg to about 10 g/day of said compound.

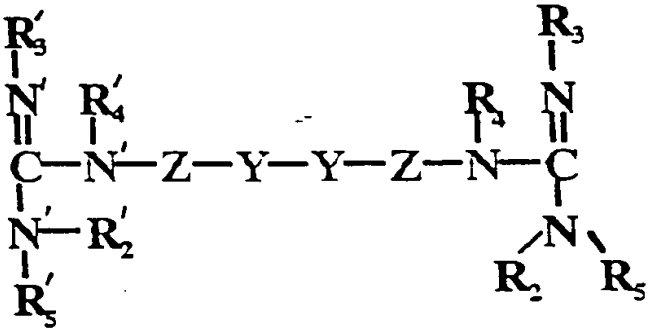
32. The composition of claim 31 formulated for administration at a dose of from about 100 mg to about 3 g/day of said compound.

33. A method for inhibiting the cytotoxic effect of peroxynitrite in a mammal comprising:

administering to the mammal an effective amount of a compound to inhibit the cytotoxic effect of peroxynitrite in the mammal, said compound having a formula selected from the group consisting of:



and



or a salt thereof, wherein:

R_2 and R'_2 are independently H, lower alkyl, alkenyl, alkylene, alkenylene, amino, aminoalkyl, hydroxy, alkoxy, thioalkylene, thioesteralkylene, phenyl or phenylalkylene, or a substituted derivative thereof;

R_3 and R'_3 are independently H, lower alkyl, alkylene, alkenylene, amino, hydroxy, thioalkylene, or a substituted derivative thereof;

R_3 and R'_3 are independently H, lower alkyl, alkylene, alkenylene, amino, hydroxy, thioalkylene, or a substituted derivative thereof;

R_1, R_5, R'_5, R_4 and R'_4 are independently H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene or amino, or a substituted derivative thereof;

Alternatively, R_1 is R_6-Y-Z- where R_6 is H, alkyl, alkenyl, phenyl, alkylene, alkenylene, phenylalkylene, acyl, $-SO_3^-$, or $-PO_3^-$, or a substituted derivative thereof, and Z and Y are as defined below;

Z and Z' are independently alkylene, alkenylene, cycloalkylene or cycloalkenylene, or a substituted derivative thereof;

Y and Y' are independently S or Se;

When R_2 or R'_2 is alkylene, alkenylene, thioalkylene, amino, hydroxy or a substituted derivative thereof, said R_2 or R'_2 may be joined to any of:

(i) R_3 or R'_3, if R_3 or R'_3 is alkylene, alkenylene or thioalkylene;

(ii) R_4 or R'_4, if R_4 or R'_4 is alkylene or alkenylene; or

(iii) R_5 or R'_5, if R_5 or R'_5 is alkylene or alkenylene;

to form 5-, 6-, or 7-membered heterocycle;

When R_2, R_3, R'_2 or R'_3 is alkylene or alkenylene, said R_2, R_3, R'_2 or R'_3 optionally may be joined to the adjacent Z or Z' to form a 5- or 6-membered heterocyclic ring, with the proviso that said

heterocyclic ring optionally being substituted with a lower alkyl, alkoxy, halo, hydroxy or amino;

When R_1 and R_4 are alkylene or alkenylene, said R_1 and R_4 optionally may be joined together to form a 5-, 6-, or 7-membered heterocycle; and

When R_1 is R_6-Y-Z-, and R_6 is alkylene or alkenylene, R_6 optionally may be joined to any of:

(i) R_2, when R_2 is alkylene, alkenylene or thioalkylene;

(ii) Z; or

(iii) R_4, when R_4 is alkylene or alkenylene;

to form a 5-, 6- or 7-membered heterocyclic ring.

34. The method of claim 33 wherein said substituted derivative of R_2, R_3, R'_2 or R'_3 is independently selected from the group consisting of one or more of alkoxy, halo, hydroxy and amino.

35. The method of claim 33 wherein said substituted derivative of R_1, R_5, R'_5, R_4 or R'_4 is independently selected from the group consisting of one or more of alkyl, alkoxy, halo, hydroxy, amino, amino alkyl (secondary or tertiary), thio and nitro.

36. The method of claim 33 wherein said R_2, R_3, R'_2 or R'_3 thioalkylene has a formula $[-(CH_2)_n-SH]$ where n is independently 1 to 4.

37. The method of claim 33 wherein said R_2 or R'_2 thioesteralkylene has a formula $[-(CH_2)_n-S-R_7]$ where R_7 is independently a lower alkyl and n is independently 1 to 4.

38. The method of claim 33 wherein said substituted derivative of Z or Z' is independently selected from the group consisting of one or more of lower alkyl, alkoxy, halo, amino, nitro and carboxyl.

39. The method of claim 33 wherein:

R_2 and R'_2 are independently selected from the group consisting of H, lower alkyl, amino, aminoalkyl, hydroxy, phenyl, phenylalkylene and a substituted derivative thereof;

R_3 and R'_3 are independently selected from the group consisting of H, lower alkyl, amino and hydroxy;

R_5, R'_5, R_4 and R'_4 are independently selected from the group consisting of H and lower alkyl;

R_1 is selected from the group consisting of H, lower alkyl and R_6-Y-Z-, where R_6 is selected from the group consisting of H, lower alkyl, acyl, $-SO_3^{--}$, $-PO_3^{--}$ and a substituted derivative thereof;

Z and Z' are independently alkylene, optionally substituted with one or more substituents selected from the group consisting of lower alkyl and carboxylic acid; and

Y and Y' are S.

40. The method of claim 33 wherein:

R_1 is selected from the group consisting of H and alkyl;

R_2 is selected from the group consisting of H, amino, hydroxy, methoxy and ethoxy;

R_3 is selected from the group consisting of H, lower alkyl and amino;

R_4 is H; and

R_5 is selected from the group consisting of H and lower alkyl.

41. The method of claim 33 wherein said compound is selected from the group consisting of aminoguanidine, hydroxyguanidine, 1-amino-2-hydroxyguanidine, 1-amino-2-methyl-2-hydroxyguanidine and diaminoguanidine.

42. The method of claim 33 wherein:

R_1 is R_6-Y-Z-;

R_6 is selected from the group consisting of H, acyl, $-SO_3^-$, $-PO_3^{--}$ and lower alkyl;

R_2 and R'_2 are independently selected from the group consisting of H and amino;

R_3 and R'_3 are independently H, amino and hydroxy;

R_4 and R'_4 are independently H, methyl and ethyl;

Z and Z' are independently alkylene optionally substituted with one or more methyl; and

Y is S.

43. The method of claim 33 wherein said compound is selected from the group consisting of mercaptoethylguanidine, mercaptopropylguanidine, S-methyl-mercaptoethylguanidine, S-methyl-mercaptopropylguanidine, 2-mercapto-2-methylpropylguanidine, mercaptoethylguanidine-S-phosphoric acid and guanidinoethyldisulfide.

44. The method of claim 33 wherein said compound reacts with the peroxynitrite, thereby inhibiting the cytotoxic effect of the peroxynitrite.

45. The method of claim 33 for inhibiting peroxynitrite-induced oxidative reactions, thereby inhibiting the cytotoxic effect of the peroxynitrite.

46. The method of claim 33 for inhibiting peroxynitrite-induced suppression of cellular respiration.

47. The method of claim 33 for inhibiting peroxynitrite-induced DNA strand breakage.

48. The method of claim 33 for inhibiting peroxynitrite-induced suppression of vascular smooth muscle contractility.

49. The method of claim 33 conducted for treating a condition where there is an advantage in inhibiting the cytotoxic effect of peroxynitrite.

50. The method of claim 49 wherein said condition is selected from the group consisting of circulatory shock, systemic inflammatory response syndrome, therapy with cytokines, ischemia-reperfusion injury of the heart, ischemia-reperfusion injury of the brain, therapy with cytokine-inducing agents, transplantation, transplant rejection, local inflammatory responses, systemic inflammation, autoimmune diseases, adult respiratory distress syndrome, arthritis, rheumatoid arthritis, diabetes mellitus, ileitis, ulcerative colitis, Crohn's disease, asthma, periodontitis, nephrosis, chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related complications, Alzheimer's disease, cardiomyopathy, adrenal insufficiency, hypercholesterolemia, atherosclerosis, bone diseases associated with increased bone resorption, pre-eclampsia, eclampsia, uremic complications, chronic liver failure, stroke, and cancer.

51. The method of claim 49 wherein said condition is selected from the group consisting of circulatory shock, myocardial ischemia and a central nervous system ischemic disorder.

52. The method of claim 33 by administering said compound by a method selected from the group consisting of oral, rectal, nasal, topical, buccal, sub-lingual, vaginal, parenteral, intramuscular, sub-cutaneous, intravenous, inhalation and insufflation administration.

53. The method of claim 33 by orally administering said compound in a pharmacologically acceptable carrier, said carrier including an ingredient selected from the group consisting of a binding agent, filler, lubricant, disintegrant, wetting agent, inert diluent, surface active agent, dispersing agent, suspending agent, emulsifying agent, edible oil, flavoring agent and mixtures thereof.

54. The method of claim 33 by topically administering in the mouth, said carrier including an ingredient selected from the group consisting of a flavor, sucrose, acacia, tragacanth, gelatin, glycerin and mixtures thereof.

55. The method of claim 33 by nasally administering said compound in a pharmacologically acceptable carrier, said carrier including an ingredient selected from the group consisting of a dispersing agent, solubilizing agent, suspending agent and mixtures thereof.

56. The method of claim 33 by administering said compound in a pharmacologically acceptable carrier by inhalation, said carrier including a propellant.

57. The method of claim 56 wherein said propellant is selected from the group consisting of dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide and mixtures thereof.

58. The method of claim 33 by administering said compound in a pharmacologically acceptable carrier by inhalation or insufflation, said carrier including an ingredient selected from the group consisting of lactose, starch and mixtures thereof.

59. The method of claim 33 by administering said compound in a pharmacologically acceptable carrier parenterally, said carrier including an ingredient selected from the group consisting of an anti-oxidant, buffer, bacteriostat, suspending agent, thickening agent, saline, water and mixtures thereof.

60. The method of claim 33 by administering said compound in a pharmacologically acceptable carrier rectally, said carrier including an ingredient selected from the group consisting of cocoa butter, polyethylene glycol and mixtures thereof.

61. The method of claim 33 wherein said compound is in combination with an ingredient selected from the group consisting of an antimicrobial agent, an immunosuppressant, a preservative and mixtures thereof.

62. The method of claim 33 wherein said compound is administered at a dose of from about 5 mg to about 17.5 g/day.

63. The method of claim 62 wherein said compound is administered at a dose of from about 5 mg to about 10 g/day.

64. The method of claim 63 wherein said compound is administered at a dose of from about 100 mg to about 3 g/day.

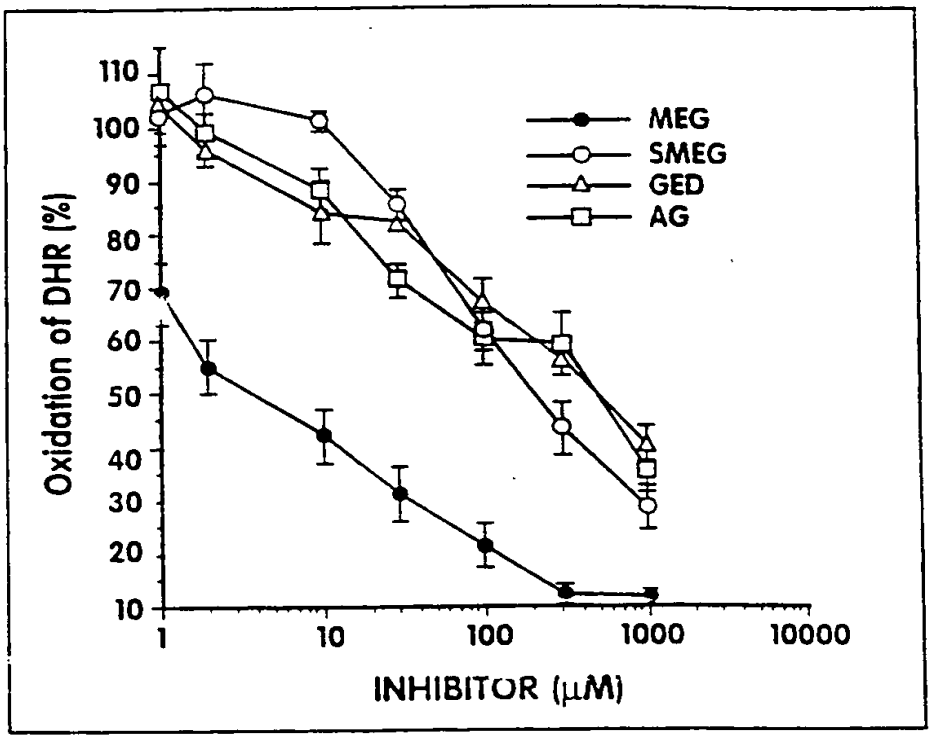

Oxidation of DHR (%)
110
100
90
80
70
60
50
40
30
20
10
1
10
100
1000
10000
INHIBITOR (μM)
MEG
SMEG
GED
AG

FIG. 1

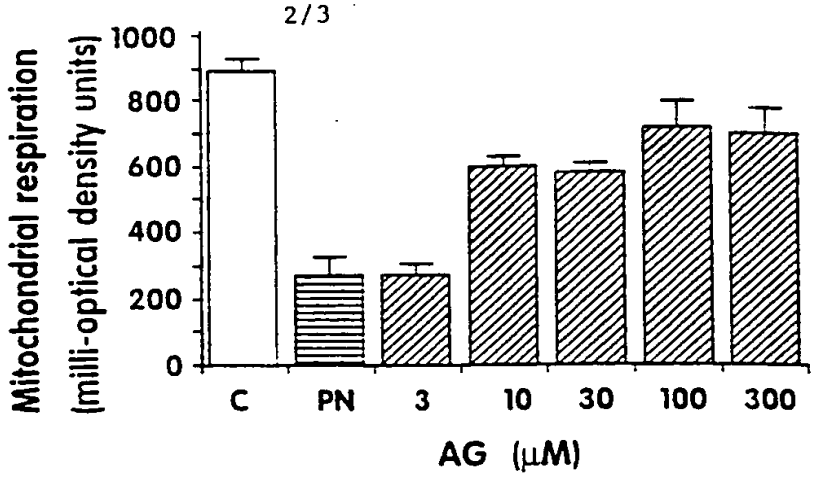


FIG. 2A

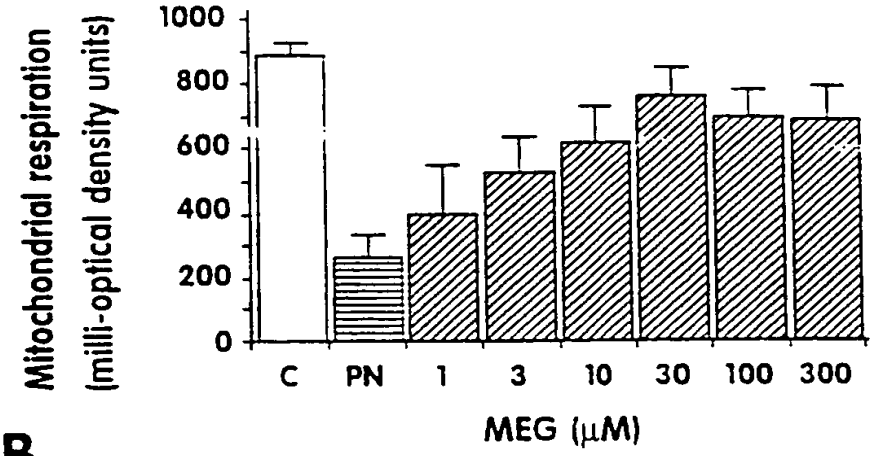


FIG. 2B

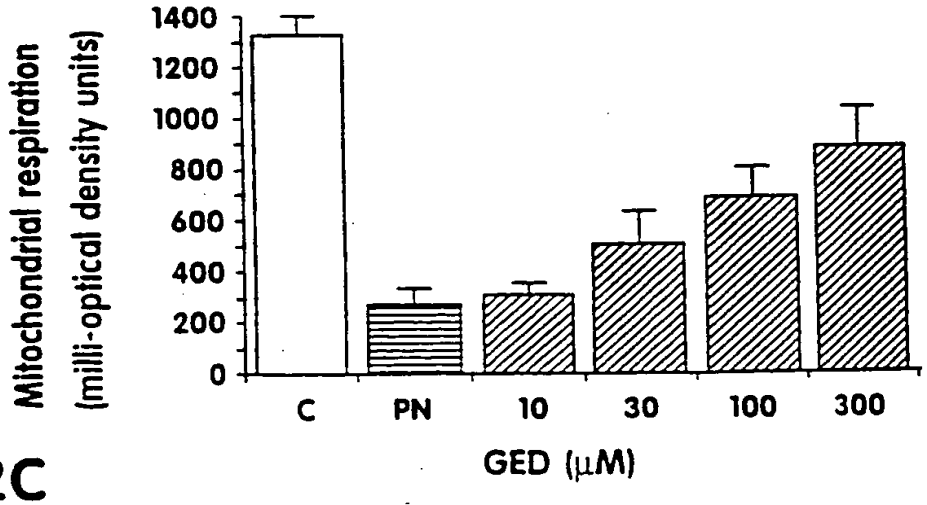


FIG. 2C

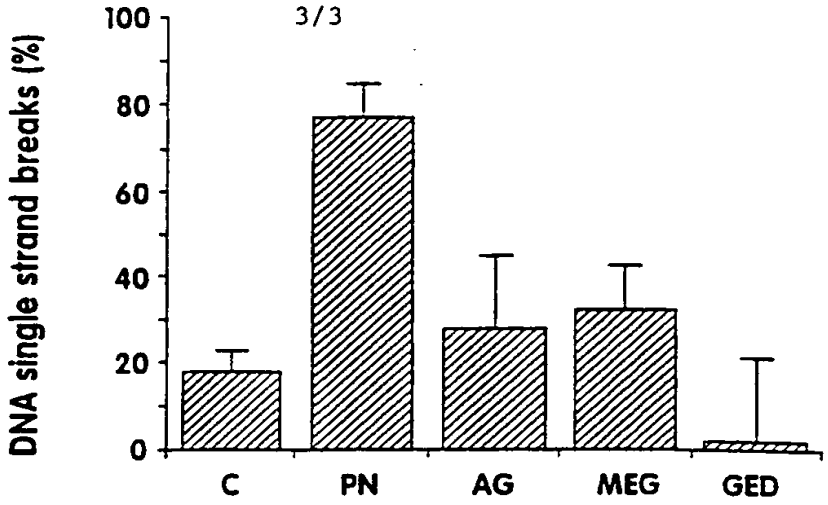

DNA single strand breaks (%)
100
80
60
40
20
0
C
PN
AG
MEG
GED

FIG. 3

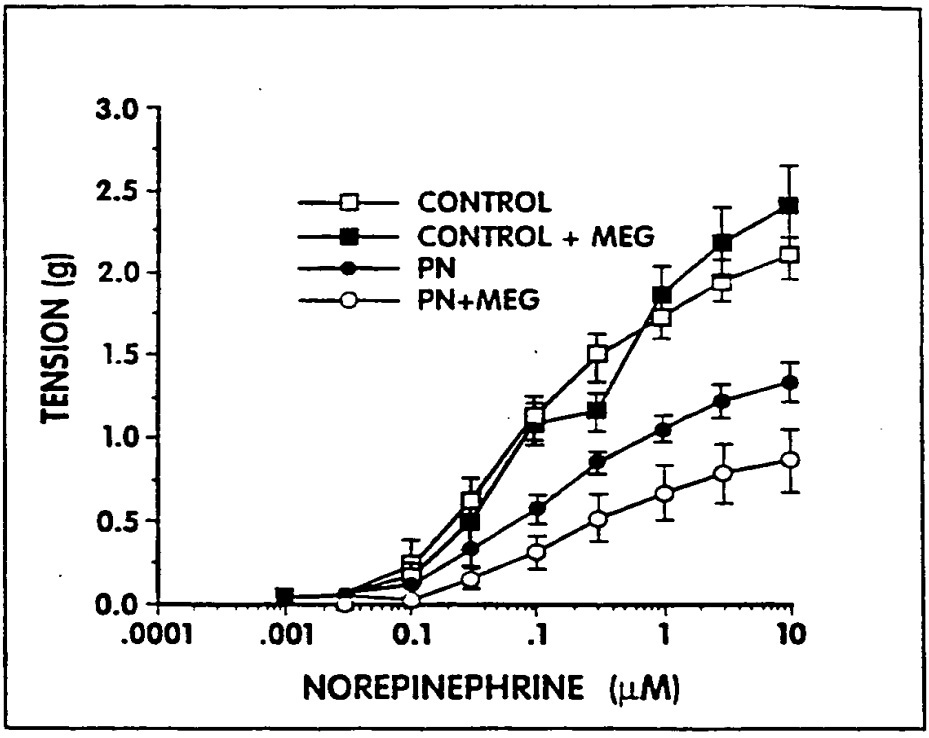

TENSION (g)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CONTROL
CONTROL + MEG
PN
PN+MEG
.0001
.001
0.1
.1
1
10
NOREPINEPHRINE (μM)

FIG. 4

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 97/08280

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/155

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 558 468 A (UNIV WASHINGTON) 1 September 1993 see page 10, line 29 - line 42; claims 1-9	1-64
X	WO 95 13805 A (UNIV DUKE ;WEINBERG J BRICE (US)) 26 May 1995 see page 5, line 14 - page 6, line 14; claims 1-13	1-64
X	WO 96 12483 A (UNIV WASHINGTON ;WILLIAMSON JOSEPH R (US); CORBETT JOHN A (US); MC) 2 May 1996 see claims 1-8	1-64
X	US 5 246 970 A (WILLIAMSON JOSEPH R ET AL) 21 September 1993 see column 2, line 5 - column 3, line 27	1-64

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 1997	22. 10. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seegert, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 97/08280

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 339 496 A (ONO PHARMACEUTICAL CO) 2 November 1989 cited in the application see claims 1-11	1-64
X	SOUTHAN G J ET AL: "SPONTANEOUS REARRANGEMENT OF AMINOALKYLISOTHIOUREAS INTO MERCAPTOALKYLGUANIDINES, A NOVEL CLASS OF NITRIC OXIDE SYNTHASE INHIBITORS WITH SELECTIVITY TOWARDS THE INDUCIBLE ISOFORM" BRITISH JOURNAL OF PHARMACOLOGY, vol. 117, no. 4, 1 February 1996, pages 619-632, XP000575067 see page 630 "Conclusions and applications"	1-64
X	SALVEMINI D. ET AL: "EVIDENCE OF PEROXYNITRITE INVOLVEMENT IN THE CARRAGEENAN-INDUCED RAT PAW EDEMA" EUR. J. PHARMACOL (NETHERLANDS), vol. 303, no. 3, 15 May 1996, pages 217-220, XP002041974 see page 219 "Discussion"	1-64
P,X	WO 96 30007 A (CHILDRENS HOSP MEDICAL CENTER) 3 October 1996 see claims 1-52	1-64
P,X	SZABO C. ET AL: "MERCAPTOETHYLGUANIDINE AND GUANIDINE INHIBITORS OF NITRIC-OXIDE SYNTHASE REACT WITH PEROXYNITRITE AND PROTECT AGAINST PEROXYNITRITE-INDUCED OXIDATIVE DAMAGE" J. BIOL. CHEM. (UNITED STATES), vol. 272, no. 14, 4 April 1997, pages 9030-9036, XP002041975 see abstract	1-64
P,X	BRAHN E. ET AL: "BENEFICIAL EFFECTS OF MERCAPTOETHYLGUANIDINE ..." FASEB JOURNAL, vol. 11, no. 3, 28 February 1997, page a530 XP002041976 see the whole document	1-64

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/US 97/08280

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0558468 A	01-09-93	US 5246971 A	21-09-93
		AT 141790 T	15-09-96
		CA 2090574 A	29-08-93
		DE 69304225 D	02-10-96
		DE 69304225 T	30-01-97
		JP 6009380 A	18-01-94
		US 5358969 A	25-10-94
WO 9513805 A	26-05-95	AU 1209995 A	06-06-95
		CA 2176747 A	26-05-95
		EP 0729356 A	04-09-96
WO 9612483 A	02-05-96	AU 4134996 A	15-05-96
US 5246970 A	21-09-93	US 5358969 A	25-10-94
		CA 2085399 A	17-06-93
		EP 0547558 A	23-06-93
		JP 5255079 A	05-10-93
		US 5246971 A	21-09-93
EP 0339496 A	02-11-89	JP 2042053 A	13-02-90
WO 9630007 A	03-10-96	AU 5319196 A	16-10-96